BIOGAS PRODUCTION FROM ANIMAL AND MODIFIED OIL PALM BUNCH WASTES

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Abstract

Increase in prices of cooking gas and the need to keep our environments clean makes research on biogas from wastes very useful. The study was carried out to use animal wastes mixed with empty bunches of oil palm fruits (EBPF) to generate gases that can be used for cooking. The cow dung was mixed with EBPF in the ratio 4:0, 4:1, 2:1 and 1:1. The microorganisms were isolated using standard method and allowed to act on it over 5 weeks at room temperature, 27^{o}C, 30 ^{o}C and 35 ^{o}C. The gases collected were subjected to laboratory analysis using Gas Chromatography (GC) with a thermal conductivity detector. The results showed that average % by volume of methane recorded for each of the ratio are 57.6±1.74 %, 52.2±1. 56 %, 25.3±2.38 % and 9.66±0. 817 % respectively. The more EBPF added the less gas of interest produced. Analysis of residue showed % N, P, K, and Mg to be 0.409±0.349 %, 0.113±0.126 %, 0.978±0.817 %, and 0.224±0.192 % respectively which implies materials that can be incorporated in soil amendment. Removing empty bunches of oil palm fruits from the environment to generate gas reduces environmental pollution caused by burning.

Keywords: Biogas, Cow wastes, Cellulose, Delignification, Oil palm waste, Residue

Introduction

The quest for the reduction of greenhouse gasses and the need for sustainable energy propelled the research interest into alternative fuels. The world is embracing renewable energy due to the depletion of fossil fuel and the adverse effect on of its use on the environment (Haider, 2020). The menace of environmental pollution and climate change has been associated with the use of fossil fuels. The developed nations have been using renewable energy resources while the developing Countries are still lacking behind (Donkin *et al*., 2013). Nigeria has abundance of animals and birds that produced wastes which are thrown into open space, causing environmental pollution and health issues in the communities. These wastes, if properly harnessed, can be source of energy for urban and rural inhabitants (Samani *et al*., 2017). Production of biogas from cow dung is preferred because energy derived from it is environmentally friendly (Putri *et al*., 2012).

Cellulosic material has wide application, such as biofuels, macromolecules, polymeric and monomeric aromatic compounds provided the lignin components is removed or extracted (Iskalieva *et al*., 2012; Varanasi *et al*., 2013;). Wood and other agricultural residues consists of cellulose, hemicelluloses and lignin (Zhang et al., 2019). Little attention was given to lignin as a compound due to its complexity and difficulties in chemical modification, however, emergence of its application is found in cosmetic, pharmaceutical, and food industries.

Biogas produced through anaerobic digestion (AD) is environmentally friendly and energy efficient, when compared to other forms of energy (Achinas *et al*., 2017).

Anaerobic digestion is more preferred because of low cost and its efficiency (Nizami and Murphy, 2010).

Generally, all kinds of biomass can be used as substrates for the biogas production, however, appropriate pretreatment, digester condition is necessary for effective biogas production (Hagos *et al*., 2017). Substrates such as animal wastes, sludge, waste waters, lignocellulosic materials, crop residue and grass silage were used (Moller *et al*., 2004; Bohn *et al*., 2007). Rumen microbes (bacteria, fungi, protozoa) are a valuable protein source and can supply 60 to 70 per cent of the animal's protein requirement. Microbiota present in the rumen of cows allowed them to digest fibers, synthesize vtamins, converts non-proteins nitrogen into protein and digest toxic substances. (Krause *et al.,* 2013).

Different researchers have also reported various species of fungi in cow dungs. Asao *et al.* (1993), reported the presence of different fungi species in the gut of ruminants and confrmed that anaerobic fungi and enzymes play a vital role in the degration and assimilation of fibrous feeds consumed by animals. Brownlee *et al.* (1996), confirm the presence of *Trichoderma reesei, Aspergillus niger* and *Penicillium italicum*, in the gut of cows using DNA and PCR to probe the fungal population while several researchers also reported the presence of fungi including *Phanerochaete chrysosporium*, *Rhizopus stolonifera*, *Mucor mucedo* and *Fusarium oxysporum* in the guts of ruminants and invariably their dungs (Azad *et al*., 2020). The ability of these fungi to digest cellulose in the grasses being fed upon by cows makes them to be in a good symbiotic relationship with the guts of cows.

Most of the biogas researchers in Nigeria used animal dung as substrate, (Ekka *et al*. 2016). A few have explored other substrates such as ornamental plant (Garba, 2002), cassava peels (Adelekan and Bamgboye, 2009), Onion bulbs, and Protein-rich biomass (pig blood) (Kovacs *et al*., 2013). Some studies have been carried out on conversion of palm oil mill wastes to useful material (Adela et al., 2014) but few focus on biogas generation especially the use of empty bunches of oil palm fruits.

Okitipupa is an agricultural based Local government where biomass wastes generation from oil palm processing, plantain peels, cassava peels and other crop residues are generated in large quantities. Animal waste such as cow dung, poultry droppings, and pig waste were also generated in substantial amount and disposed on land surfaces. All these can be harnessed into a useful source of renewable energy. The burning of empty bunches and other solid wastes constitute air pollution. There is need to find a way of converting these wastes to a valuable resource. The objective of the study is to convert empty bunches of oil palm fruits to biogas thereby circumventing burning which is the usual practice in the area.

Materials and Methods

Materials used include fresh cow dung and empty palm fruit bunches.

Waste Collection: Fresh cow dung are collected from Agric Reality Farm at Maclean village off Aye road, Okitipupa, Ondo State, Nigeria. The empty palm fruit stalk bunches were collected from an Oil mill factory located in Ayeka, Igbodigo, Okitipupa, Ondo State. The cow dungs were collected using sterile polythene bags immediately they are excreted, labelled, and taken to the laboratory for analysis.

Bacteria and Microbial screening: Spread plate technique was used for the isolation and different culture characteristics were observed after incubation. Total plate count of bacteria in each sample was determined using a colony counter and calculated as colony forming units per ml (CFU/ml) with the formula:

CFU/ml = No. of colonies x Dilution factor/ volume of inoculum.

Streak plate method was used and the isolated colonies were individually sub cultured to obtain pure culture. Differential tests and biochemical test was carried out. Biochemical test used to identify the isolates are catalase test, citrate test and urease test. Isolated fungal colonies were identified for both microscopic and macroscopic examinations (McGinnins and Borgers, 1980).

Pretreatment of empty oil palm bunches (EBPF): The empty bunches of oil palm fruits were dried and milled to reduce the particle size. Delignification was done by boiling the oil palm wastes in 1.5M NaOH for 2 hours. It was cooled to room temperature, filtered washed with IL of hot distilled water. The solid were dried in an oven for 48 hours at 45 ^{o}C milled into powder. Delignification by alkaline pretreatment with 2 - 4% NaOH in autoclave at 121°C has been suggested (Pramasari *et al*., 2021; Lourenço *et al*., 2021).

Slurry preparation: The modified method used by Ahamed *et al*. (2016), was adopted for calculation. The slurry of total weight 2 kg was prepared by weighing 500g of cow wastes and 140g of delignify empty bunches of oil palm fruits (EBPF) while1360g of water was added. The cow wastes, EBF and water represented 25 %, 7% and 68% of the total slurry respectively to achieve 4: 1 of waste to EBF ratio. The weight was adjusted to obtain other ratio used for the experiment.

Digestion and biogas generation: The batch digester was set while the connection were tightly fitted to prevent air leakage. The material remained in the digester throughout the entire digestion period, no new fresh substrate was added and no digest residue was removed during the process. The methane yield was calculated from % volume recorded. The assumption adopted was that, at standard temperature and pressure, 1g of oxygen demand take 400mL (0.4 m^3 required 1kg of oxygen demand) of methane (Hamilton, 2022).

Analysis of the gas and the residues:

Gas Chromatography – Mass Spectrometry (GC-MS) (Perkin Elmer) was used to determine different components of gases produced with a thermal conductivity detector (TCD). Atomic Absorption Spectroscopy (AAS) (Bulk Scientific model) was used for determination of metals in the residue. Heavy metal contents (Cd, Mn, Ni, Zn, Cu, Fe) in the biogas residues were determined using Atomic Absorption Spectroscopy (AAS) (APHA, 1998).

Results and Discussion

The results of bacterial loads on cow dungs are presented (Table1). Gut of ruminants have been widely reported to accommodate various microflora because the foregut of ruminants houses an ecosystem of micro-organisms that breakdown plant cell wall. It allows the animal to obtain nutrients from both the plant material and the microbes themselves (Abecia *et al*., 2013). The gram positive bacteria are stained dark blue, while gram negative bacteria appeared pink red.

Table 1: Microbial Loads on cow dungs

S/N	Stock	10^{-3}	10^{-5}
1	175 x 10^7	12.0	3.0
2	167 x 10^7	12.0	3.0
3	205 x 10^7	14.0	3.0
4	190 x 10^7	13.0	4.0
5	165 x 10^7	12.0	4.0
6	180 x 10^7	13.0	4.0
7	155 x 10^7	12.0	3.0
8	160 x 10^7	12.0	3.0
9	105 x 10^7	11.0	3.0
10	165 x 10^7	13.0	4.0

Bacillus cereus, *Escherichia coli*, *Clostridum perfringes*, *Pseudomonas aeruginosa Salmonella sp*, *Staphylococcus aureus*, *Streptomyces thermos-autotrophicus*, and *Alcaligenes faecalis* were identified and isolated from the cow dung used in the present study (Table 2). This is in agreement with previous studies (Hawkes *et al.*, 2008). Different species of Bacillus such as *Escherichia coli*, *Proteus mirabilis*, *Streptomyces spp*, *Pseudomonas sp*, *Salmonella sp*, *Staphylococcus aureus*, and *Alcaligenes faecalis* have been isolated from cow dungs (Kartikey *et al.*, 2015; Adeyemo and Waleola 2016; Cole *et al*., 2022).

Table 2: Results of Biochemical tests on bacterial isolates from cow dungs

Isolate	Gram test	Form	Cat	Cit	Ure	Oxi	Lac	Glu	Suc	Mal	Suspected Organism
1	+	Rod	+	+	+	+	+	+	Variable	+	*Bacillus cereus*
2	-	Rod	-	-	-	-	+	+	Variable	+	*Escherichia coli*
3	+	Rod	-	+	-	-	+	+	+	+	*Clostridum perfringes*
4	-	Rod	+	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
5	-	Rod	+	+	-	-	-	+	-	-	*Salmonella sp*
6	+	Cocci	+	+	-	+	+	+	-	+	*Staphylococcus aureus*
7	+	Filament	+	+	+	+	+	+	+	+	*Streptomyces thermoautotrophicus*
8	-	Rod	+	-	-	+	+	+	+	-	*Alcaligenes faecalis*
9	-	Rod	+	+	+	-	-	-	-	-	*Proteus mirabilis*

Key: Gram test – Gram staining test, Form- shape under microscopic view, Cat – Catalase test, Cit – Citrate test,Ure- Urease test, Oxi- Oxidase, Lac- Lactose, Glu- Glucose, Suc- Sucrose, Mal- Maltose, + = positive, - = negative, -/+ = variable.

The Clostridium genus is involved in hydrolysis of the substrates, acidogenesis and acetogenesis stages of anaerobic digestion (Tapadia - Maheshwari *et al*., 2019). *Escherichia coli* were found in lactose and glucose and maltose but variable in sucrose. The few presence of E. coli is due to killing effect of anaerobic digestion. This is consistent with findings of Ye *et al*. (2012). Furthermore, increase in temperature can easily destroy E. Coli and caused its rapid death (Liang *et al*., 2021).

With the exception of the first preparation without EPFB, other slurry was prepared such that the total weight of the slurry was 2 kg. With the assumption of Hamilton, (2022), that 1 kg of oxygen demand (OD) take 400 mL of methane and 1kg OD remove 0.4m^3 of methane produced. Fifty percent (50 %) cow dung with no EPFB and 50 % of water yielded 2.86 x 10^{-3}m^3 of methane (Table 3). Twenty-five percent (25 %) cow dung with 7 % EPFB and 68 % of water yielded 2.61x 10^{-3}m^3 of methane. Twenty-five percent (25 %) cow dung with 12.5 % EPFB and 62.5 % of water yielded 1.26 x 10^{-3}m^3 of methane. Twenty-five percent (25 %) cow dung with 25% EPFB and 50 % of water yielded 0.483 x 10^{-3}m^3 of methane.

Table 3: mass of slurry with quantity of materials used

Cowdung (g)	EPBF (g)	Cow dung: EPBF	Water (g)	Methane yield m^3 (x10-3)
500	0	4:0	500	2.86
500	140	4:1	1360	2.61
500	250	2:1	1350	1.26
500	500	1:1	1000	0.483

Significantly high percentage of methane (57.6±1.74) was recorded when 100% of cow dung was used (4:0) (Table 4). The composition of biogas and the intensity of gas formation are heavily depending on the quantity of the animal wastes, viability of micro-organisms present in the waste and temperature (Odonkor and Mahami, 2020).

Table 4: Results of gas produced at different ratio of cow wastes with EBPF

Cow Dung : EBPF	CH_4 (% volume)	NH_3 (% volume)	CO (% volume)	H_2S (% volume)	CO_2 (% volume)
4:0	57.6 ±1.74c	0.209 ±0.045b	0.718 ±0.075d	0.796 ±0.036c	30.85 ±1.20d
4:1	52.2 ±1.56c	0.048 ±0.038a	0.439 ±0.0376c	0.409 ±0.052b	21.4 ±0.832c
2:1	25.3 ±2.38b	0.015 ±0.006a	0.241 ±0.022b	0.169 ±0.031a	8.55 ±0.42c b
1:1	9.66 ±0.817a	0.016 ±0.007a	0.047 ±0.01a	0.106 ±0.0147a	0.387 ±0.22a

Note: Data with the same letter down the column are not significantly different using Duncan Multiple Range Test.

There is no significant difference in % methane produced when 4:1 of cow wastes to empty bunches of oil palm wastes was used and 100 % of cow wastes (4:0) was used at the temperature of 33^{0}C and pH range of 6.8 to 7.5. This agrees with pattern of results reported when sheep manure was used without modification (Yoshida *et al*., 2020). The % volume of gases produced decreases as the quantity of cow dung reduces which agrees with the report of Putri *et al*. (2012). The pH of the slurry was varied between 5 and 7.5, it was found that higher gas pressure was recorded at the lower pH (Figure1). This is consistence with findings of Jacob *et al*., (2018). The activity of methanogenic bacteria is more favoured by acidic condition, which also depends on when the animal was fed last (Madigan *et al*., 2011). Alkaline pH is toxic to the bacteria which decreases the activity due to conversion of ammonia to ammonium, hence lower gas pressure was recorded (Chen et al., 2008).

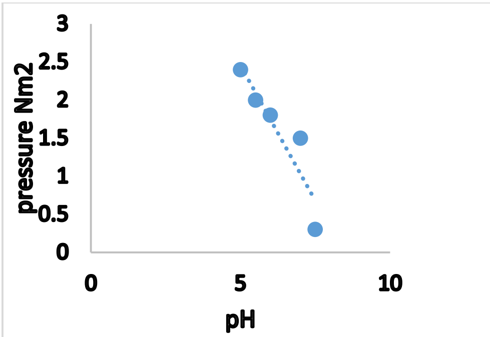

Figure1: Variation of pH with gas pressure.

In this experiment, three temperatures 27^0C, 30^0C and 35^0C were used corresponding to the laboratory environment, under the shed constructed and outside the shed. The gas pressure recorded increases with increase in temperature (Figure 2). At 27^0C, the gas pressure of 1.05Nm^2 was measured, at 30^0C, the gas pressure of 1.6 Nm^2 was recorded while at 35^0C, the pressure of 2.05Nm^2 was recorded. Most methanogenic bacteria are mesophilic and thrive in conditions that resemble their original habitat which is cow rumen
(Mussoline *et al*., 2013).

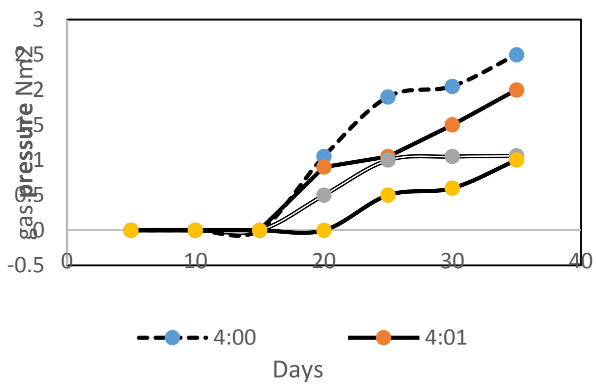

Figure 2: A graph of pressure monitored at different days for different preparations

No visible changes was recorded in the pressure gauge until 20th day after incubation. With 100 % cow-dung (4:0), the pressure recorded are 1.05 Nm^2, 1.9 Nm^2, 2.05 Nm^2, and 2.5 Nm^2 for day 20, 25, 30 and 35 respectively (Figure 2). When cow-dung with EBPF in the ratio 4: 1 was used, the pressure recorded are 0.9 Nm^2, 1.05 Nm^2, 1.5 Nm^2, and 2 Nm^2 for the days respectively. With the cow-dung and EBPF in the ratio 2:1, the pressure recorded are 0.5 Nm^2, 1 Nm^2, 1.05 Nm^2, and 1.06 Nm^2 for the days respectively. Finally, with cow-dung and EBPF in the ratio 1: 1, the pressure recorded are nil, 0.5 Nm^2, 0.6 Nm^2, and 1 Nm^2 for the days respectively.

The average concentration of N, P, K and Mg in the residue are 0.409 ± 0.349 %, 0.113 ± 0.126 %, 0.978 ± 0.817 %, and 0.224 ± 0.192 mg/kg respectively (Table 5). Liang *et al*. (2021) recorded 0.059 %, 0.05% and 0.052% for N, P and K respectively for animal wastes fermentation residue.

Table 5: Elemental composition of residues of digested cow dung with EBPF

Elements	Min	Max	Mean ± SD
N (%)	0.001	0.97	0.409±0.349
P(%)	0.001	0.38	0.113±0.126
K(%)	0.01	2.26	0.978±0.817
Mg (mg/kg)	0.007	0.59	0.224±0.192
Mn (mg/kg)	0.001	0.814	0.247±0.26
Zn(mg/kg)	0.001	0.621	0.209±0.21
Fe (mg/kg)	3	129	64.4±45.7
Cu (mg/kg)	0.01	0.867	0.341±0.269
Cd (mg/kg)	0.001	0.011	0.005±0.004
Ni(mg/kg)	0.003	0.204	0.099±0.08

The appreciable percentage of N, P and K found in the residue is comparable with previous findings in literature (Panjaitan, *et al*., 2022). High concentration of K and Mg found in cow dung is responsible for high activity of methanogens (Abu -Ashour *et al*., 2010). This makes the residue a material that can be recommended for possible use as soil amendment.

Mn concentration ranged between 0.001 to 0.814 mg/kg with the mean value of 0.247 ± 0.26 mg/kg. Zn concentration ranged between 0.001 to 0.621 mg/kg with the mean value of 0.209 ± 0.21 mg/kg. Fe concentration ranged between 3 to 129 with the mean value of 64.4 ± 45.7 mg/kg. Cu concentration ranged between 0.01 to 0.867 mg/kg with the mean value of 0.341 ± 0.269 mg/kg. The elemental analysis of the residue showed the presence of some toxic elements such as Ni and Cd. Ni concentration ranged between 0.003 to 0.204 mg/kg with the mean value of 0.099 ± 0.08 mg/kg (Table 3). Cd concentration ranged between 0.001 to 0.011 mg/kg with the mean value of 0.005 ± 0.004 mg/kg. The presence of toxic elements in the residue showed that it can be used to land fill waste dump site in a professional way in order to prevents its migration into groundwater.

Conclusion

Results from the present study showed that cow dung can best be combined with EBPF in the ratio not exceeding minimal use of EBPF at acidic pH and moderate temperature. The EPBF can be used generate biogas for domestic use and avert health hazard arising from its open burning. The residue contains substantial amount of N, P, and K which suggest possible suitability for soil amendment to improve crop yield. The use of EBPF to generate biogas can remove waste from our environments and improve energy yield for sustainable development. Further study should be carried out on the use of residue for fertilizer to ascertain a particular crop it can support.

Conflict of Interest: There is no conflict of interest

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